Nature 303:818-823 because Boulter et al. teaches DNA of rat origin that encode α_3, α_4 and β_2 rat neuronal nicotinic acetylcholine receptor subunits, but not human nicotinic acetylcholine receptors; and Schofield, et al., Grenningloh et al. and Noda et al. allegedly show that isolation of DNA encoding a human ligand-gated ion channel subunit from **an appropriate** library using a probe from another mammal was routine, and that genes among this superfamily were known to be highly conserved, thereby indicating that one of ordinary skill in this art would have had a reasonable expectation of success.

It is concluded that because:

> an artisan of molecular biology knew that the value of neuroreceptor research such as that described in the Boulter et al. publication would ultimately lie in its applicability to human subjects, the artisan would have found it obvious to have isolated nucleic acids encoding human homologs of the rat nicotinic acetylcholine receptor subunits by probing a human neuronal library with nucleic acid probes encoding rat subunits using those methods described in each of Grenningloh et al., Schofield et al., and Noda et al.

This rejection is respectfully traversed.

As stated in the previous response, which arguments are incorporated by reference herein, it is respectfully submitted that this rejection is defective in at least three aspects: (1) none of the cited references teaches or suggests that subunits homologous to the rat subunits exist in humans; (2) none of the cited references teaches or suggests anything regarding rat α_2 subunit; (3) as demonstrated in the DECLARATION and discussed below the references fail to teach appropriate library; and (4) as demonstrated in the DECLARATION, the cited references do not provide a reasonable expectation of success. As described in the DECLARATION, following the teachings of the references does not necessarily produce expected results nor, in the case of α_4, permit successful isolation of DNA encoding subunits that correspond to the rat subunits.

As stated in the previous response, claims to chemical compounds, such as the instantly claimed DNA molecules are held to be prima facie obvious only when there evidences of very close structural similarities between the a claimed compound and a prior art compound. Absent such showing prima facie has not been established. See, e.g., In re Grabiak, 226 USPQ 870, 871 (Fed. Cir. 1985), In re May, 197 USPQ 601 (CCPA 1978), In re Wilder, 195 USPQ 426, 429 (CCPA 1977), In re Hoch 166 USPQ 406 (CCPA) 1970 (acid, methyl ester), In re Papesch 50 CCPA 1084, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

Also, as stated in the previous response:

> the existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious, in the absence of other prior art that suggests the claimed DNAs. . . . **There must, however, still be prior art that suggests the claimed compound in order for a prima facie case of obviousness to be made out; as we have already indicated, that prior art was lacking here with respect to claims 5 and 7. Thus, even if, as the examiner stated, the existence of general cloning techniques, coupled with knowledge of a protein's structure, might have provided motivation to prepare a cDNA or made it obvious to prepare a cDNA, that does not necessarily make obvious a particular claimed cDNA.** "Obvious to try" has long been held not to constitute obviousness. In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1680-81 (Fed. Cir. 1988). A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out. Thus, Maniatis's teachings, even in combination with Bohlen, fail to suggest the claimed invention [In re Deuel (CAFC, 1995).

The Examiner has failed to set forth a prima facie case of obviousness.

> **The combination of teachings of the cited references does not teach or suggest any of the claimed DNA fragments, cells, subunits or methods, and does not provide a reasonable expectation of success of achieving the desired goal of cloning the claimed human neuronal NAChR subunits.**

As discussed in detail in the previous response, none of the cited references or applicant's alleged admission teaches or suggests anything

regarding the properties of the claimed human neuronal NAChR subunits. In addition, none of the cited references or applicant's alleged admission, singly or in any combination thereof, teaches or suggests an enabling methodology for cloning human neuronal NAChR subunits or DNA encoding such subunits. Finally, as the attached DECLARATION and the discussion in the previous response, there was no reasonable expectation of success that DNA encoding human subunits could have been cloned nor were the results achieved those that were expected prior to having cloned the DNA encoding the selected subunits.

As described in the instant specification and in the DECLARATION, a significant problem to be solved in order to clone DNA encoding the human neuronal nicotinic acetylcholine receptor subunits was identifying and obtaining suitable source(s) of cDNA to probe for the desired sequences. Human brain tissue samples must be obtained post-mortem and must be obtained soon after death in order to minimize autolysis and degradation of the nucleic acids.

The claimed DNA fragments were isolated using rat neuronal acetylcholine receptor subunit DNA fragments as probes in numerous human cDNA libraries. Neuronal nicotinic acetylcholine receptor are not uniformly expressed throughout the brain and are expressed at very low levels. In addition, as described in the DECLARATION, libraries prepared from RNA from brain tissue that would have been expected to contain mRNA encoding a particular subunit, based on the expression of such subunit in corresponding rat brain tissue, did not contain cDNA that hybridized to the corresponding rat probe. Consequently, it was necessary to probe numerous human cDNA libraries, including pre-frontal cortex cDNA, parietal cDNA, temporal cortex cDNA, brain stem cDNA, basal ganglia cDNA, and spinal cord cDNA, to obtain

and identify various fragments of DNA encoding the human neuronal subunits. Then, after partial sequencing and restriction mapping of several such fragments, composite DNA sequences for the human neuronal α_2, α_3 and β_2 were deduced. Similar strategies, however, failed to yield human clones encoding the α_4 and α_5 subunits.

Thus, because of the very low concentration of various human neuronal subunits in the human neuronal tissue, the frequently very localized presence of some of the human neuronal subunits in various sources of tissue, the difficulty in obtaining human neural tissue, including brain tissue, with which to work, as well as the high level of care necessary to ensure the presence of intact mRNA in the source human neuronal tissue, there was no reasonable expectation that DNA encoding any or all of the human subunits could have been obtained. Furthermore, after screening numerous libraries, it was only possible to obtain DNA encoding the human α_2, α_3 and β_2 subunits.

For example, as discussed in the DECLARATION, based on information regarding the distribution of receptor subtypes in the rat, such as that taught by Wada et al. ((1989) J. Comp. Neurol. 284: 314-335), indicating that α_4 is expressed at high levels in the rat thalamus, a human thalamus cDNA library was screened with DNA encoding the rat α_4 NAChR subunit as a probe in order to obtain DNA encoding the human α_4 subunit. Unexpectedly, however, the hybridizing clones, Th 2.1, Th 2.111 and Th 2.13 (see Fig. 4 of the application) do not encode the human α_4 subunit but do encode the α_2 subunit. In addition, the regions of clones Th 2.1 and 2.13 that were sequenced are only about 80% homologous to the rat α_2 gene and the regions of the Th 2.111 clone that were sequenced were only about 75% homologous the rat α_2 gene. Furthermore, as described in the DECLARATION and discussed below, applicant has not as yet successfully isolated DNA encoding a human neuronal NAChR α_4 subunit.

Absent the instant application, one of ordinary skill in the art would not have had a reasonable expectation of successfully cloning the α_3 subunit from a

library derived from peripheral nervous system tissue. Furthermore, IMR32 cells express few, if any functional nicotinic acetylcholine receptors (see, e.g., Gotti et al. ((1986) Biochem. Biophys. Res. Commun. 137: 1141-1147, and Clementi et al. (1986) J. Neurochem. 47: 291-297, which is of record in this application). As described in the DECLARATION, however, twenty-four clones, including a full-length clone, were isolated from the IMR32 cell library. Thus, the mRNA encoding the α_3 subunit appears to be quite abundant in IMR32 cells, even though it has been reported that receptors are rare.

Thus, in view of the failure of the prior art to teach a source of α_3-encoding cDNA or to provide insights regarding the structure of the human gene, one of ordinary skill in the art would not have had a reasonable expectation of success that DNA encoding the human α_3 subunit could have been obtained.

As taught by the prior art, see, e.g., Deneris et al. (1988) Neuron 1:45-54, which will be provided in connection with a supplemental IDS submitted under separate cover, a functional channel is formed from α and β subunits. As described in the DECLARATION, IMR32 libraries, which contain high levels of α-encoding DNA contain very low levels of β_2 encoding DNA. Thus, in light of the cited art, which suggests that α and β are co-expressed in rat tissues, one of ordinary skill in the art would have expected that, if abundant RNA encoding an α subunit is present in a particular cell type, that abundant β-encoding RNA would also be present. DNA encoding the β_2 subunit was, however, obtained from a prefrontal cortex library, despite teachings in the prior art that β is not expressed at high levels in the prefrontal cortex.

In light of the same prior art, applicant did not obtain full-length clones encoding the human α_4 subunit or the human α_5 subunit. As described in the DECLARATION, at least two unsuccessful attempts were made in order to isolate DNA encoding the human α_4 subunits. Rat α_4 DNA was used to screen

a human brain stem cDNA library under low stringency and a habenula cDNA library under conditions of high stringency. Eighteen clones in the brain stem library hybridized to the probe. Some of the hybridizing clones encoded an α_3 subunit, but none of the hybridizing clones, however, encoded an α_4 subunit. Similarly, none of the hybridizing clones in the habenula library encoded an α_4 subunit, although one clone encoded an α_2 subunit.

In order to isolate DNA encoding the human α_5 subunit, a randomly primed library prepared from RNA isolated from dibutyryl cAMP-induced IMR32 cells was screened under conditions of high stringency with a 1.1 kb fragment including the 5' end of the coding portion of rat α_5-encoding DNA. No hybridizing clones were obtained. Chini et al. (1992) using a probe from the 3' end of the obtained clones encoding the human α_5 subunit. Clearly none of the prior art suggested to applicant an enabling methodology for cloning the α_5 subunit.

To the extent that the art does not provide an enabling methodology for cloning DNA encoding subunits that applicant was not successful in cloning, it does not provide an enabling methodology for cloning DNA encoding subunits that applicant successfully cloned. Thus, to the extent the prior art does not provide an enabling methodology for isolation of DNA encoding the α_4 and α_5 subunits, it does not provide an enabling methodology for isolation of DNA encoding the α_2, α_3 and β_2 subunits.

Therefore, merely because the prior art teaches DNA encoding subunits from a related species, rat, does not necessarily lead to the conclusion that one of ordinary skill in this art will have a reasonable expectation of successfully cloning DNA encoding human subunits. None of the cited references teaches or suggests anything regarding the corresponding human subunits. The art does

not teach sources of such subunits nor enabling methodology for selecting probes that might be successfully used to screen any library. Only in light of the instant specification, which teaches such clones, would one of ordinary skill in the art have been able to have used the cited art to have obtained DNA encoding the human α_2, α_3, and β_2 subunits.

The fact that the method of isolation may be routine is not a proper basis for rejection of a product

In addition, in view of the recent CAFC decision In re Deuel, U.S. App. LEXIS 6200 (Fed. Cir. 1995), as well as other cases, including Amgen and In re Bell, it is now clear that rejections based on the method of purification are improper. In Deuel the issue on appeal was:

> whether the combination of a prior art reference teaching a method of gene cloning, together with a reference disclosing a partial amino acid sequence of a protein, may render DNA and cDNA molecules encoding the protein prima facie obvious under §103.

The CAFC held that:

> Because Deuel claims new chemical entities in structural terms, a prima facie case of unpatentability requires that the teachings of the prior art suggest the claimed compounds to a person of ordinary skill in the art. Normally a prima facie case of obviousness is based upon structural similarity, i.e., an established structural relationship between a prior art compound and the claimed compound. . . Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologs because homologs often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties. Similarly, a known compound may suggest its analogs or isomers, either geometric isomers (cis v. trans) or position isomers (e.g., ortho v. para).

> In all of these cases, however, the prior art teaches a specific, structurally-definable compound and the question becomes whether the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention. See In re Jones, 958 F.2d 347, 351, 21 USPQ2d 1941, 1944 (Fed. Cir. 1992); In re Dillon, 919 F.2d 688, 692. . . .
>
> The fact that one can conceive a general process in advance for preparing an undefined compound does not mean that a claimed specific compound was precisely envisioned and therefore obvious. A substance may indeed be defined by its process of preparation. That occurs, however, when it has already been prepared by that process and one therefore knows that the result of that process is the stated compound. The process is part of the definition of the compound. But that is not possible in advance, especially when the hypothetical process is only a general one.

Deuel is on point with respect to this rejection which is based on a general process that could possibly have been used to identify (not even isolate) a protein whose existence had not yet been demonstrated. The instantly claimed DNA fragments are previously undefined chemical compounds. The prior art in this instance does not suggest the resulting chemical compounds. As discussed above, prior to isolating the instantly claimed DNA, it was not known nor was it predictable from the cited references what the structure of DNA encoding human nicotinic acetylcholine receptors would be nor what properties of the resulting subunits, whether they are similar or different from rat, would be. In fact, as described in the previous DECLARATION of record and in the specification, some properties are similar and some are different. Such similarities and differences are each indicative of the unobviousness of the DNA.

There is no teaching or suggestion in any of the cited references of modifications of the rat nicotinic acetylcholine receptor-encoding DNA that would have resulted in the instantly claimed DNA. The precise differences are not taught or suggested by any of the cited references.

Following the teachings of the cited references, one of ordinary skill in the art might have suggested that all of the subunits would be 97-99% homologous to rat and all would exhibit similar distributions in the human brain as in the rat brain, but they do not. Thus, the instantly claimed DNA fragments and cells are not prima facie obvious over the cited references.

DECLARATION OF ELLIS

This is substantially an updated (with respect to the present employment of Mr. Ellis) DECLARATION that is of record in the parent application, which is now U.S. Patent No. 5,369,028. This DECLARATION and above discussion demonstrate that there was no reasonable expectation of success that DNA human subunits could have been cloned. It was not possible to successfully clone all of the subunits that applicant attempted to clone and the particular results achieved were not suggested by the cited art. In addition, the cited art provides no indication of which parameters are critical and provides no direction as to which choices are likely to be successful so that the success that was achieved was accomplished by varying all parameters and trying each of numerous possible choices until full-length clones encoding several of the subunits were eventually isolated or constructed. Contrary to the assertion of the Examiner, as described in the attached DECLARATION, isolation of such DNA was not straightforward despite art describing DNA encoding rat nicotinic acetylcholine receptors nor were the results achieved predictable or expected.

As described in the instant specification and in the DECLARATION, a significant problem to be solved in order to clone DNA encoding the human neuronal nicotinic acetylcholine receptor subunits was identifying and obtaining suitable source(s) of DNA to probe for the desired sequences. Human brain tissue samples must be obtained post-mortem and must be obtained soon after death in order to minimize autolysis and degradation of the nucleic acids.

* * *

In view of the above remarks and the amendments and remarks in the previous response, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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